Bifunctional Rhodium Intercalator Conjugates as Mismatch-directing DNA Alkylating Agents

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Supporting Information

Synthesis and characterization of conjugate **1** and intermediates

Synthesis and characterization of conjugate 1 and intermediates

Conjugate **1** of $[Rh(phen)(chrysi)(bpy)]^{3+}$ tethered to an aniline mustard was prepared in a thirdteen step sequence as outlined in Schemes S1 to S4.

$[Rh(phen)(chrysi)(NH_3)_2]^{3+}$ **2** was synthesized in four steps from rhodium trichloride, phenanthroline, and 5,6-chrysenequinone, and purified as described by Mürner, Jackson, and Barton (Scheme S1).[1]

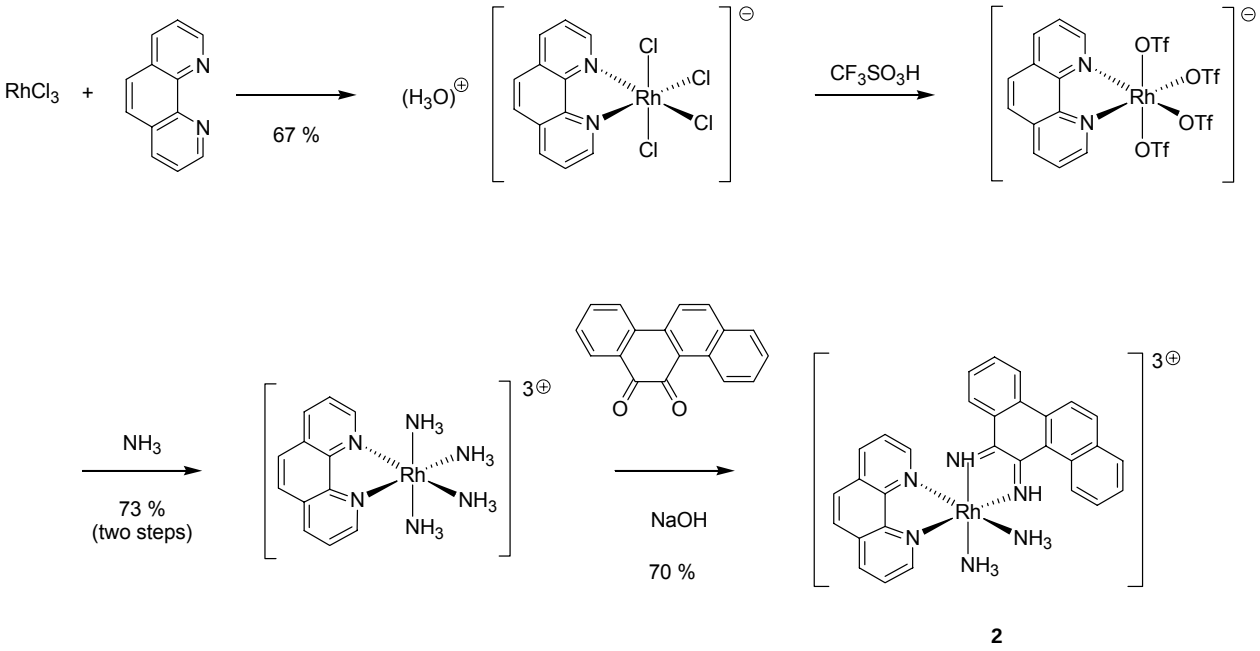
The synthesis of the aminoalkyl-functionalized bipyridine **6** is shown in Scheme S2. The mono-anion of 4,4'-dimethylbipyridine, obtained by deprotonation of **3** with one equivalent of freshly prepared lithium diisopropylamine, was reacted with an excess of 1,6-dibromohexane under carefully controlled conditions.[2, 3] After aqueous workup and column chromatography on silica with a gradient of dichloromethane and diethylether, 4-(7-bromoheptyl)-4'-methylbipyridine **4** was obtained in 58 % yield. This was converted to phthalimide **5** in almost quantitative yield by reaction with potassium phthalimide followed by cleavage to 4-(7-aminoheptyl)-4'-methylbipyridine **6** with hydrazine monohydrate in methanol.[4]

Aminoalkyl-substituted bipyridine **6** was then reacted with $[Rh(phen)(chrysi)(NH_3)_2]^{3+}$ **2** in anhydrous acetonitrile at elevated temperature to give $[Rh(phen)(chrysi)(bipy^{\#})]^{3+}$ **7** in 43 % yield after purification on Sephadex SP C25 (Scheme S3).

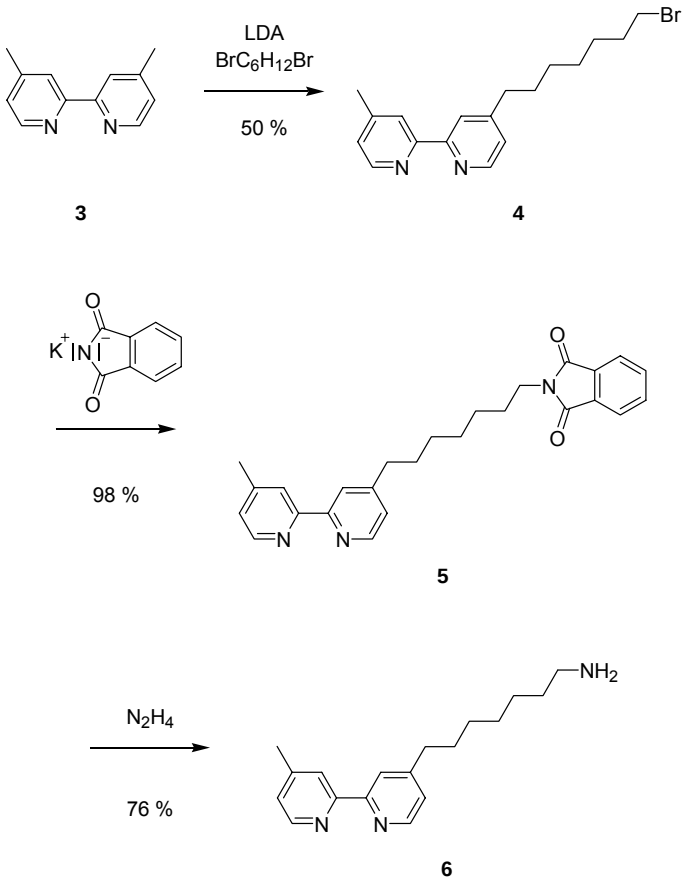
The aniline mustard **12** was prepared in four steps from 4-aminophenol **8** as outlined in Scheme S4.[5-7] The starting material was reacted with bromoacetic acid ethyl ester and separated from other alkylation products by column chromatography on silica to give **9**. This compound was then reacted with oxirane to give (4-(*N*,*N*-bis(2-hydroxy-ethyl)-phenoxy)acetic acid ester **10**. The hydroxy groups were substituted by chlorides upon treatment with phosphoroxytrichloride in benzene to give **11** and the ester protective group then removed under acidic conditions to yield **12**.

Conjugate **1** was then prepared by coupling of **7** with **12** in anhydrous dimethylformamide in the presence of EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and HOBt (1-hydroxybenzotriazol) as shown in Scheme 1 and finally purified by semi-preparative HPLC.

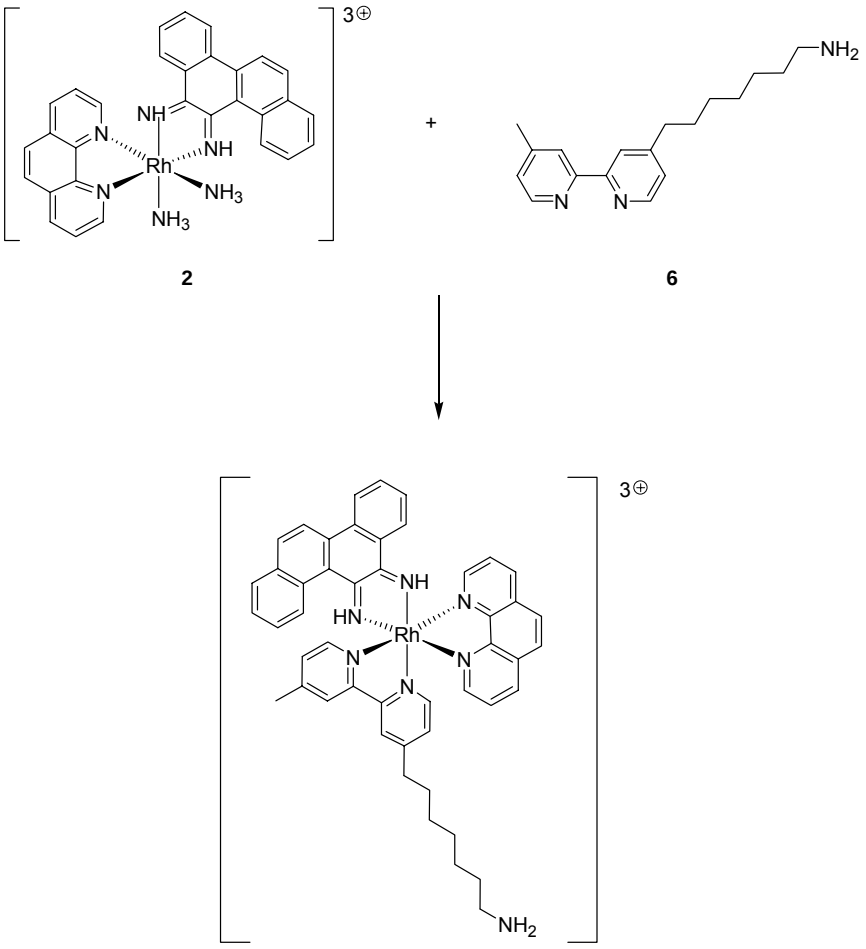
Scheme S1. Synthesis of tris-heteroleptic rhodium complexes.



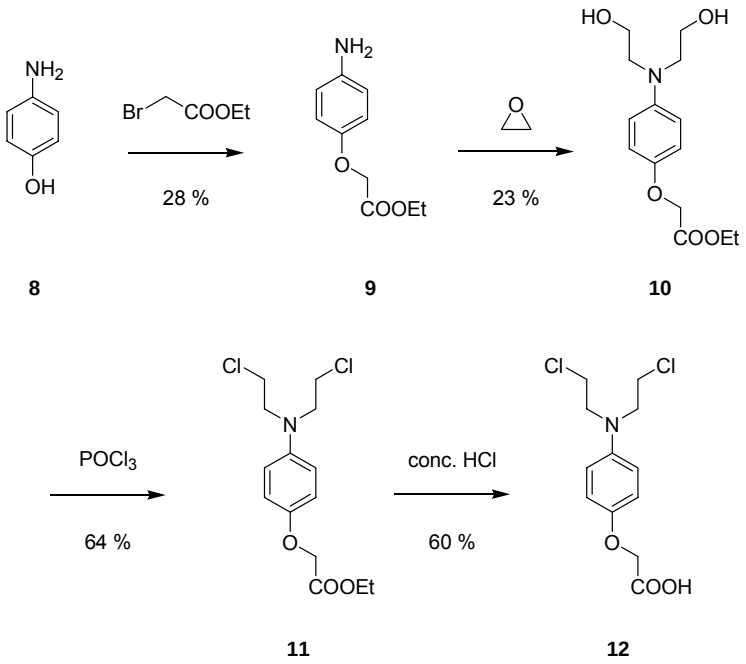
Scheme S2. Synthesis of an aminoalkyl-functionalized bipyridine ligand.



Scheme S3. Introduction of the aminoalkyl-substituted bipyridine to the rhodium-chrysenequinone diimine complex.



Scheme S4. Synthesis of a carboxy-functionalized aniline mustard.



Synthetic procedures and characterization

4-(7-Bromoheptyl)-4'-methylbipyridine 4

Anhydrous tetrahydrofuran (25 ml) was placed in a dry Schlenk bottle under argon and cooled to -78 °C with an acetone/dry ice mixture. Then diisopropylamine (4.6 ml, 32.8 mmol) was added via syringe followed by *n*-butyllithium (15 % solution in hexane, 17.2 ml, 27.4 mmol) to give a slightly yellow solution which was kept at -78 °C. In a separate Schleck bottle, 4,4'-dimethylbipyridine (5.0 g, 27.2 mmol) was dissolved in anhydrous tetrahydrofuran (150 ml), precooled to -78 °C and then added to the former solution. The mixture, which turned deep brown, was stirred at that temperature for 1 h and then allowed to warm to 0 °C by placing in an ice bath. In another Schlenk bottle, 1,6-dibromohexane (20 ml, 51.2 mmol) was dissolved in anhydrous tetrahydrofuran (20 ml) at room temperature and then added to the former solution at once(!). The mixture first turned blue but the color changed to yellow within 1.5 h hours, after which the reaction was quenched by addition of water (100 ml). After stirring for 30 min, the pH was adjusted to pH = 5 and the mixture left to stand overnight. The yellow organic phase was separated, the aqueous phase extracted with ether (100 ml) and dichloromethan (2x 100 ml), and the combined organic phases washed with 5 M sodium chloride, dried over magnesium sulfate, and the solvent removed *in vacuo* at room temperature(!). The slightly yellow oil obtained was purified by column chromatography on silica first with dichloromethane as the eluent to remove excess 1,6-dibromohexane, and then with a gradient of dichloromethane/ether (100:0 → 0:100). After removal of the solvent, the product was obtained as a white solid (5.48 g, 15.78 mmol, 58.0 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.55 (t, *J* = 4.8 Hz, 2H), 8.23 (m, 2H), 7.13 (d, *J* = 4.8 Hz, 2H), 3.40 (t, *J* = 6.7 Hz, 2H), 2.69 (t, *J* = 7.8 Hz, 2H), 2.44 (s, 3H), 1.84 (m, 2H), 1.70 (m, 2H), 1.37 (m, 6H); MS(ESIpos): 369 (M+Na), 347 (M+H), 267 (M-Br).

4-(7-phthalimidoheptyl)-4'-methylbipyridine 5

4-(7-Bromoheptyl)-4'-methylbipyridine **4** (0.70 g, 2.00 mmol) was dissolved in anhydrous dimethylformamide (125 ml) and solid potassium phthalimide (0.45 g, 2.40 mmol) was added to the slightly yellow solution. After stirring for 90.5 h, the solution was diluted with chloroform (80 ml) and then poured into water (250 ml). The phases were separated and the aqueous phase extracted with chloroform (2x 100 ml). The combined organic phases were washed with 0.2 M sodium hydroxide (100 ml), water, and brine, dried over magnesium sulfate, and the solvent removed to give the product as a colorless oil which solidified to a white solid upon standing (0.81 g, 1.96 mmol, 98.0 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.53 (m, 2H), 8.21 (m, 2H), 7.83 (m, 2H), 7.69 (m, 2H), 7.12 (m, 2H), 3.67 (t, *J* = 7.2 Hz, 2H), 2.67 (t, *J* = 7.8 Hz, 2H), 2.43 (s, 3H), 1.66 (m, 4H), 1.36 (m, 6H); MS(ESIpos): 414 (M+H), 436 (M+Na).

4-(7-aminoheptyl)-4'-methylbipyridine 6

Phthalimide **5** (0.81 g, 1.96 mmol) was dissolved in methanol (50 ml) by heating to 70 °C to give a colorless solution. Then hydrazine monohydrate (0.2 ml, 4.1 mmol) was added and the reaction mixture stirred at 70 °C for 19 h. The solvent was removed and 6 M hydrochloric acid (100 ml) added to the white solid obtained. The suspension was ex-

tracted with chloroform, the organic phase washed with 6 M hydrochloric acid (60 ml), and the combined aqueous phases then adjusted to pH = 8 with 5 M sodium hydroxide. The aqueous phase was extracted with chloroform and ether, the combined organic phases dried over magnesium sulfate and evaporated to dryness to give the product as a white solid (420 mg, 1.48 mmol, 76 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.54 (t, *J* = 4.5 Hz, 2H), 8.21 (m, 2H), 7.13 (d, *J* = 4.8 Hz, 2H), 2.68 (m, 4H), 2.43 (s, 3H), 1.85 (s, 2H), 1.70 (m, 4H), 1.39 (m, 6H); MS (ESIpos): 284 (M+H).

[Rh(phen)(chrysi)(bipy$^{\#}$)]Cl_3 7

4-(7-aminoheptyl)-4'-methylbipyridine **6** (160.0 mg, 0.56 mmol) was dissolved in anhydrous acetonitrile (35 ml) and then [Rh(phen)(chrysi)$(NH_3)_4$]Cl_3 **2** (226.6 mg, 0.33 mmol), dissolved in 25 ml of the same solvent with sonication, was added to the former solution. The mixture was heated to reflux under argon for 19.5 h, cooled to room temperature, and the solvent removed to give a deep brown solid. The solid was dissolved in a minimum amount of 0.05 M magnesium chloride and methanol and then loaded onto a column of Sephadex SP C25 (20x4 cm) pre-equilibrated with 0.05 M magnesium chloride and the product eluted with a gradient of 0.05 → 0.5 M magnesium chloride. All magnesium chloride solutions were acidified with a drop of hydrochloric acid. The main fraction was loaded on a C_{18} Waters SepPak (10 g) which was washed with cupious amount of water, and the product then eluted with acetonitrile containing a drop of trifluoroacetic acid. In order to obtain a more pure product (> 98 % from HPLC), the purification procedure was repeated once. After removal of the solvent in a stream of air and drying *in vacuo*, the product was obtained as a red-brown solid (131.4 mg, 0.13 mmol, 39.4 %). MS (ESIpos): 820 (M^+-3Cl-2H), 410 (M^{2+}-3Cl-H); UV/Vis (water): 212, 264, 298 (sh), 312 (sh), 398 (6200) nm (l mol^{-1} cm^{-1}); HPLC (100:0 →30:70 water/acetonitrile over 50 min) > 98 %.

(4-Aminophenoxy)acetic acid ethyl ester 9

4-Aminophenol **8** (20.0 g, 183.3 mmol) was dissolved in ice-cold degassed anhydrous dimethylformamide (250 ml) under argon and then solid sodium hydride (55 - 65 % in oil, 7.6 g, 190 mmol) was added in small portions. The solution was stirred at 0 °C for 30 min after which it had turned deep red-brown. Then bromoacetic acid ethyl ester (20.2 ml, 182.2 mmol) was slowly added at 0 °C via syringe and the resulting mixture stirred at room temperature overnight. After removal of the solvent, the resulting orange gum was dissolved in a mixture of ethylacetate and water (250 ml, 1:1), the organic phase separated, the aqueous phase extracted with ethyl acetate (2x 100 ml), the combined organic phases washed with sodium hydrogencarbonate, brine, and water, drived over magnesium sulfate and the solvent removed. The resulting brown oil was purified twice by column chromatography on silica with chloroform/methanol (9:1) as the eluent to give the product as an orange oil which slowly solidified (9.83 g, 50.4 mmol, 27.5 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.76 (d, *J* = 9.3 Hz, 2H), 6.62 (d, *J* = 9.0 Hz, 2H), 4.53 (s, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 1.29 (t, *J* = 7.2 Hz, 3H); MS (ESIpos): 196 (M+H), 218 (M+Na).

(4-Bis(2-hydroxyethyl)aminophenoxy)acetic acid ethyl ester 10

(4-Aminophenoxy)acetic acid ethyl ester **9** (7.89 g, 40.4 mmol) was dissolved in a degassed mixture of acetic acid and water (35 ml, 1:1) under argon to give a orange-to-red solution which was cooled to 0 °C. Oxirane (9.10 g, 206.6 mmol) was condensed into a separate Schlenk bottle immersed in acetone/dry ice and then added with a syringe precooled to -20 °C to the former solution. The mixture was allowed to warm to room temperature while stirring under argon for 18.5 h and the deep brown solution then concentrated in vacuo at < 40 °C. Water (25 ml) was added to the resulting oily material and the product extracted with ether (4x 100 ml). The combined organic phases were carefully washed neutral with 2 M sodium hydrogencarbonate (100 ml) in a large separation funnel (strong effervescence!) followed by water, dried over magnesium sulfate and the solvent then removed at < 40 °C. The resulting material was twice recrystallized from toluene/hexanes (300 ml, 1:1) to remove some brown oil and then purified by column chromatography on silica with chloroform/methanol (9:1) as the eluent. The product was obtained as a slightly pinkish oil which very slowly solidified to a white solid (2.58 g, 9.1 mmol, 22.5 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.82 (d, *J* = 9.0 Hz Hz, 2H), 6.66 (d, *J* = 9.0 Hz, 2H), 4.53 (s, 2H), 4.24 (q, *J* = 7.1 Hz, 2H), 3.76 (t, *J* = 5.0 Hz, 4H), 3.45 (t, *J* = 4.8 Hz, 4H), 1.28 (t, *J* = 7.1 Hz, 3H); MS (ESIpos): 284 (M+H), 306 (M+Na).

(4-Bis(2-chloroethyl)aminophenoxy)acetic acid ethyl ester 11

(4-Bis(2-hydroxyethyl)aminophenoxy)acetic acid ethyl ester **10** (0.81 g, 2.86 mmol) was dissolved in anhydrous benzene (10 ml) and then phosphoroxy trichloride (0.8 ml, 8.58 mmol) was added via syringe followed by heating to 100-110 °C for 1 h. Afterwards, the solution was poured on ice, the organic phase separated, dried over magnesium sulfate, and shaken with activated alumina. After removal of the solvent, the product was obtained as a colorless oil which solidified to a white solid (0.58 g, 1.81 mmol, 63.3 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.84 (d, *J* = 9.0 Hz, 2H), 6.62 (d, *J* = 9.3 Hz Hz, 2H), 4.52 (s, 2H), 4.24 (q, *J* = 7.2 Hz, 2H), 3.59 (m, 8H), 1.27 (t, *J* = 7.2 Hz, 3H); MS (ESIpos): 320 (M+H).

(4-Bis(2-chloroethyl)aminophenoxy)acetic acid 12

(4-Bis(2-chloroethyl)aminophenoxy)acetic acid ethyl ester **11** (0.62 g, 1.94 mmol) was dissolved in concentrated hydrochloric acid (10 ml), heated to reflux for 1 h and cooled to room temperature. After addition of water (50 ml), the product was extracted into diethylether (4x 50 ml), the combined organic phases wahsed with water, dried over magnesium sulfate, and the solvent removed to give a white solid (0.34 g, 1.16 mmol, 59.8 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.87 (d, *J* = 9.0 Hz, 2H), 6.65 (d, *J* = 9.3 Hz, 2H), 4.60 (s, 2H), 3.62 (m, 8H); MS (ESIpos): 292 (M+H).

[Rh(phen)(chrysi)(bipy)]Cl_3 1

[Rh(phen)(chrysi)(bipy$^{\#}$)]Cl_3 **7** (5.8 mg, 6.2 µmol) was dissolved in anhydrous dimethylformamide under argon to give a bright orange solution. Then, (4-Bis(2-chloroethyl)aminophenoxy)acetic acid **12** (2.0 mg, 6.8 µmol) was added as a solid followed by HOBt (1-hydroxybenzotriazol, 2.1 mg, 15.5 µmol). Finally, EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, 5 µl, 36.7 µmol) was added and the solution stirred at room temperature for 2.5 h. Then, water (5 ml) was added and the resulting solution loaded on a C_{18} Waters SepPak (5 g) which was washed with cupious amount of water. The product was eluted with acetonitrile containing a drop of trifluoroacetic acid, the solvent removed in a stream of air and the resulting red-brown solid stored at -80 °C. The product was then dissolved in 600 µl of acetonitrile and purified by semi-preparative HPLC on a HP/Agilent 1100 system with a Dynamax 300 Å C_1 column using a gradient of 0.5 % trifluoroacetic acid in water and acetonitrile (100:0 → 30:70 over 60 min). The main peak was collected, the solution frozen at -80 °C, and the product lyophilized to dryness. The resulting red-brown solid was dissolved in water (2 ml) and kept at -80 °C. Aliquots were taken from the thawed solution and diluted as needed. MS (ESIpos): 1093 (M^+-3Cl-2H).

Literature

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